REMARKS

Claims 6-8, 10, 20, 31-37 and 48 are pending. Claims 1-5, 9, 11, 17-19, 21, 23-30, and 38-47 have been cancelled without prejudice. Claims 6-8, 10, 20, and 31-37 have been amended. However, the amendments to and/or the cancellation of the claims were made solely to expedite prosecution of the present application. Support for the amendments can be found throughout the application as originally filed. No new matter has been added.

Compliance with Sequence Rules

The Examiner states that the application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821 (a)(1) and (a)(2), but that have not been accorded sequence identifiers. The Applicants provide herewith a computer readable copy of the Sequence Listing, a substitute paper copy of the Sequence Listing, and a statement under 37 CFR §1.821(f) stating that the content of both are the same. The amendments to the specification were made to insert sequence identifiers following sequence disclosures and replace the original Sequence Listing with an amended version. The Applicants respectfully submit that the application is now in compliance with 37 CFR 1.821 through 1.825. No new matter has been added.

Rejection of Claims Under 35 U.S.C. §112, first paragraph

The Examiner rejected claims 1-11, 17-21, and 24-47 under 35 U.S.C. §112, first paragraph, asserting that "the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims." According to the Examiner,

> Claims 1-11, 17-21, and 24-47 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for vectors and host cells comprising either SEQ ID NO:1, SEQ ID NO:1 with N182Q and N263Q mutations, or fragments of these nucleic acids encoding antigenic polypeptides; methods of making the polypeptides encoded by these nucleic acids; and for transgenic mammals which comprise these nucleic acids in their germ line operably linked to a transcription control sequence which causes their transcription in a mammary gland of the mammal, wherein the mammal

express in a mammary gland the polypeptides encode by these nucleic acids, and secretes the polypeptides into milk, does not reasonably provide enablement for any other nucleic acid; for transgenic animals which do not secrete MSP-1 in their milk; or for any DNA vaccine.

Claims 1-5, 9, 11, 17-19, 21, 23-30, and 38-47 have been cancelled without prejudice, thereby obviating the Examiner's rejection to these claims. The remaining claims, as amended, are directed to transgenic non-human mammal whose genome includes a modified nucleic acid encoding a parasite protein or fragment thereof operably linked to a promoter for expression in the mammary gland and methods of producing a parasitic protein or fragments thereof in the milk of such mammals. Based on the knowledge in the art and the guidance provided in the present application, a skilled artisan could clearly make and use the invention as claimed without undue experimentation.

The present invention is based, in part, on the discovery that expression of a parasitic protein, namely MSP-1, and variants thereof could be obtained in the milk of transgenic mammals by lowering the AT-content and/or eliminating AUUUA mRNA instability motifs of a naturally occurring parasite protein-encoding sequence. Using codons which are preferentially expressed in the mammary gland to modify the parasitic protein-encoding sequence, Applicants were able to increase the level of expression of the parasitic protein from a non-detectable level with the sequence as it naturally occurs in the parasite to levels of 1mg/ml or greater using the modified nucleic acid sequence.

Applicants have provided guidance as to the preferred mammary gland promoters, preferred mammary gland-specific codons, methods of modifying a parasite protein encoding sequence to reduce the AT-content, and methods of eliminating AUUUA mRNA instability motifs from a parasite protein-encoding sequence. Applicants have also provided guidance on the production of transgenic mammals that express such parasitic proteins and have demonstrated that such mammals do express the parasitic protein in milk.

In addition, sequences encoding several parasitic proteins other than MSP-1 were known in the art at the time of filing. For example, the following references disclose sequences encoding various antigens associated with *Plasmodium*: U.S. Patent Numbers 5,543,323; 5,225,534; 5,395,614; 5,646,247; 5,194,587; and PCT Publication Number WO 91/18922.

Using the methods provided in the present application, a skilled artisan would clearly be able to modify any of the known parasitic protein sequences to reduce its AT-content and/or eliminate AUUUA mRNA instability motifs.

There is also sufficient guidance provided in the present application for a skilled artisan to prepare a transgenic mammal for expression such modified parasite protein encoding sequences. Moreover, Applicants have demonstrated that use of such modified sequences does result in expression of a parasite protein in the milk of a transgenic mammal. Therefore, undue experimentation would not be required to practice the claimed invention.

For the reasons discussed above, Applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claims Under 35 U.S.C. §112, second paragraph

The Examiner rejected claims 4-11, 17-21, 26-42, 43, and 45-47 under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In particular, the Examiner states that claims 4-11, 17-21, 26-42, 45, and 47 are indefinite because "they recite 'a preferred mammary tissue-specific codon'". The Examiner asserts that the phrase is not adequately defined by the claims or the specification. Further, the Examiner states that

> it is unclear what comparison is implied by this definition. The definition could refer to codons used more prevalently by mammary cells than by either yeast cells or bacterial cells. Because yeast and bacteria have different codon preferences, the set of codons used more prevalently by mammary cells than bacterial cells is likely to be different than the set of codons used more prevalently than yeast cells. So, it is unclear what set of codons is intended.

Claims 1-5, 9, 11, 17-19, 21, 23-30, and 38-47 have been cancelled without prejudice thereby obviating the Examiner's rejection to these claims. The remaining claims have been amended to recite a "preferred mammary gland-specific codon". Thus, the claims as amended do not encompass, as the Examiner asserts, "all cells found in mammary tissue including adipose cells, lymphocytes, red blood cells, etc.", but do encompass cells of the mammary gland.

Further, Applicants respectfully traverse the Examiner's assertion that it is unclear what comparison is implied by the definition. On page 7, starting at line 28 of the present application,

the specification provides that "Each of these features are achieved by replacing one or more codons of the natural gene with codons recognizable to, and preferably preferred by the cell system that encode the same amino acid as the codon which was replaced in the natural gene." It is clear from this definition that the term "preferred mammary gland-specific codon" refers to codons that are used more prevalently by mammary gland cells than other codons. This is also consistent with the art-known meaning of "preferred codon." At the time of filing, the term "preferred codon" was used throughout the art to refer to a codon which is preferentially used by a particular cell system over other codons encoding the same amino acid. See, for example, Kotula et al. (1991) Biotechnology 9(10): 1386-1389 (submitted herewith as Exhibit A) discussing optimizing codons for those preferred in yeast, and Urdea et al. (1983) PNAS USA 80 (24): 7461-7465 (submitted herewith as Exhibit B) discussing "yeast-preferred codons." Thus, based on its definition in the present application and its known meaning in the art, it is clear that the term "preferred mammary gland-specific codon" refers to those codons preferentially expressed by mammary gland cells as compared to other codons encoding the same amino acid.

Therefore, Applicants respectfully request that the Examiner withdraw this rejection.

The Examiner also rejected claims 23-25, 38-41, 43, and 46 as indefinite because they recite the phrase "nucleic acid of a bacterial, viral, or parasitic protein." Claims 23-25,38-41, 43 and 46 have been cancelled without prejudice thereby obviating the Examiner's rejection to these claims.

For the reasons provided above, Applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claims Under 35 U.S.C. §102 (a)

Claims 1-11, 17-19, 23, 24, 27-29, 31-33, 35-40, and 44-47 have been rejected by the Examiner under 35 U.S.C. §102 (a) "as being anticipated by Longacre-Andre et al. (WO 97/30159)." The Examiner states

> It is noted that the methods of the instant invention are directed to preparing a nucleic acid for expression in a mammalian cell, whereas the nucleic acid of Longacre-Andre is expressed in an insect cell. Absent evidence to the contrary, the nucleic acid of Longacre-Andre is considered to expressible in a mammalian cell, particularly in view of the codon optimization which increases the GC content of the nucleic acid. Because the

vector comprising the nucleic acid of Longacre-Andre is foreign to the insect cell in which the nucleic acid is expressed, the vector is considered to be transgenic relative to the insect cells. Thus Longacre-Andre anticipates the claims.

Applicants respectfully traverse this rejection. Claims 1-5, 9, 11, 17-19, 21, 23-30, and 38-47 have been cancelled without prejudice, thereby obviating the Examiner's rejection to these claims. The remaining claims, as amended, are directed to a transgenic non-human mammal whose genome includes a modified nucleic acid encoding a parasite protein or fragment thereof operably linked to a promoter for mammary gland expression and methods of producing a parasitic protein or fragments thereof in the milk of such mammals.

Longacre-Andre discloses a recombinant C-terminal fragment of MSP-1 of the merozoite form of *Plasmodium falciparum* protein expressed in a baculovirus system. Longacre-Andre does not teach or suggest using modifications to obtain expression of the protein in a mammalian cell, and does not teach or suggest replacing codons specifically to achieve expression in the mammary gland. In fact, Longacre-Andre make no suggestion whatsoever of expression in mammary gland cells or any methods which would achieve expression in these cells. Moreover, Longacre-Andre provide absolutely no suggestion that expression in a baculovirus system should, or could, be translated to expression in mammary cells. Therefore, Longacre-Andre clearly do not teach each and every element of the claim. Thus, Longacre-Andre cannot anticipate the claimed invention, and the Applicants respectfully request that the Examiner withdraw this rejection.

Rejection of Claims Under 35 U.S.C. §102 (e)

Claims 23, 24, 38, 39, 40, and 46 are rejected by the Examiner under 35 U.S.C. §102(e) "as being anticipated by Seed et al. (U.S. Patent 5795737, issued 8/18/98)." Claims 23, 24, 38, 39, 40, and 46 have been cancelled without prejudice, obviating the Examiner's rejection of these claims.

Rejection of Claims Under 35 U.S.C. §103(a)

Claims 1-11, 17-21, 23, 24, 27-34, 38-41, and 44-47 have been rejected by the Examiner under 35 U.S.C. §103(a) "as being unpatentable over Dziegiel et al. (U.S. Patent Number

5,231,168), Seed et al. (U.S. Patent Number 5,795,737), Akashi et al. (1994) *Blood* 83(11):3182-3187, and Bosch et al. (U.S. Patent Number 5,736,131)." According to the Examiner,

> Dziegiel teaches an expression vector comprising a nucleic acid encoding an antigen of *Plasmodium falciparum*. See abstract. The expression vector may be used in mammalian cells for the purpose of producing and isolating the antigen, and may be used to construct transgenic animals which express the antigen. See column 18, lines 54-65; and column 19, lines 61-63. The GC content of the nucleic acid is about 30%. See column 16, lines 40-43. Prior to use of the expression vector, the nucleic acid may be modified by silent nucleotide substitutions which favor the codon usage of the organism in which the nucleic acid will be expressed. See column 20, lines 66 to column 21, line 7; and column 21, lines 36-40. The nucleic acid comprises at least two ATTTA motifs within the coding region. See bases 962-966, and bases 1896-1900. Dziegiel does not specifically recommend reducing the AT- content of the nucleic acid, the removal of the mRNA instability motifs, or the introduction mammary tissue-specific codons.
>
> Seed teaches that codon optimization may be used to increase expression of foreign genes in mammalian cells. See column 1, lines 8-10; and column 2 lines 7-11. Preferred codons are always those with the highest possible GC content. See lines 33-37, and Table 1, bridging columns 7 and 8. Seed does not use the phrase "mammary tissue-specific codons", however, for the amino acids A, R, N, Q, H, I, L, K, P, F, and S, Seed teaches that the most preferred codon is the same codon which Applicant chose to use most frequently, as evidenced in Fig. 3A. For this reason, it is concluded that Seed teaches the "mammary tissue-specific codons" for these amino acids. Seed also teaches avoiding the inclusion of AUUUA sequences in synthetic genes. See column 12, lines 35-37.
>
> Bosch teaches removal of mRNA instability motifs from nucleic acids which are to be expressed in heterologous hosts. Bosch also teaches that codon optimization is advisable. See column 4, lines 12-21.
>
> Akashi teaches that the function of AUUUA mRNA destabilization motifs is not restricted by their location within the mRNA. These motifs need not be located in the 3'-untranslated region of mRNAs, and are capable of destabilizing mRNAs even when located within the coding region. See abstract and Fig. 1.
>
> It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the nucleotide sequences of Dziegiel by decreasing its AT-content and removing mRNA destabilization motifs. One would have been motivated to do so because Seed teaches that, of all the codons encoding a given amino acid, the preferred codon for expression in mammalian cells is the one with the highest GC-content. One would have been motivated to remove mRNA-

destabilizing motifs from the coding region of the nucleic acids because Akashi teaches that these sequences can be active in the context of the coding region, and because Bosch and Seed suggest that mRNA-destabilizing motifs should be removed from sequences to be expressed in heterologous hosts. One would have been motivated to remove the ATTTA sequences from the coding region of Dziegiel because Akashi teaches that these sequences can destabilize mRNAs even when located within the coding region. By using the preferred codons of Seed in the construction of any synthetic gene, one would apparently inherently use what Applicant refers to as "mammary tissue-specific codons", because Seed suggests the same codons used by applicant for 14 of the 18 amino acids which have more than one codon. Thus the invention as a whole was *prima facie* obvious.

Claims 1-5, 9, 11, 17-19, 21, 23-30, and 38-47 have been cancelled without prejudice, thereby obviating the Examiner's rejection with regard to these claims. Applicants respectfully transverse the rejection of the remaining claims as amended.

The claims, as amended, are directed to a transgenic non-human mammal whose genome includes a modified nucleic acid encoding a parasite protein or fragment thereof operably linked to a promoter which directed expression in mammary gland cells and methods of producing a parasitic protein or fragments thereof in the milk of such mammals.

Dziegiel et al. disclose an expression vector comprising a nucleic acid encoding GLURP, an antigen of *Plasmodium falciparum*. It appears that the Examiner has cited Dziegiel because GLURP is a *Plasmodium falciparum* antigen having a high AT content and AUUUA destabilization motifs, and because Dziegiel suggests generally that sequence modifications can be made for various purposes. As conceded by the Examiner, however, Dziegiel does not teach nor suggest reducing the AT-content of the nucleic acid, nor the removal of mRNA instability motifs, nor the introduction of mammary gland-specific codons. In addition, Dziegiel does not teach or suggest expression of the parasitic proteins in the mammary gland of transgenic animals.

Moreover, Dziegiel does not address, nor even suggest that there would be any problems associated with expression of a parasite protein. For example, there is no discussion of any difficulty in obtaining recombinant parasitic proteins in expression systems. Thus, Dziegiel provides no motivation to modify a parasite protein to obtain its

expression in the milk of a transgenic mammal.. As the Examiner has conceded that Dziegiel teaches none of the elements of the present invention, therefore, Applicants submit that the reference bears no relevance to the patentability of the remaining claims.

Seed et al. disclose the use of codon optimization to obtain increased expression of eukaryotic and viral proteins in eukaryotic cells. The preferred codons cited in Seed are based upon human genes that are highly expressed. There is no teaching or suggestion in Seed that preferred codons should be selected based upon mammary tissue codon usage. Moreover, several of the codons taught to be preferred by Seed et al. are different than those codons preferred in mammary tissue of a non-human mammal. There is no teaching whatsoever in Seed et al. to express parasitic proteins in the milk of a transgenic mammal.

In addition, Seed, like Dziegiel, fails to address suggest any problems involved in obtaining recombinant parasitic proteins in expression systems, and, in particular, the difficulty in expressing parasitic proteins in mammary gland cells. The Examiner stated in the Response to Arguments section,

> Applicant argues that there is no suggestion in Seed that naturally occurring parasitic proteins would not be expressed in mammalian cells, but does not explain why this is necessary for the purpose of the rejection. It is not required by the claims.

The determination of whether Seed addresses or acknowledges this problem is essential for a proper obviousness analysis. There must be some suggestion or motivation that one of skill in the art could glean from Seed to make the invention claimed by the Applicants. Absent teaching or suggestion by Seed et al. or one of the other references cited, no such motivation can exist. Since Seed does not suggest that naturally occurring parasitic proteins would not be expressed in mammalian cells, one of skill in the art would have no motivation to use Seed to arrive at the Applicants' claimed invention.

Bosch et al. disclose hybrid toxins derived from portions of *Bacillus thuringiensis* insecticidal crystal proteins. In the column and lines pointed out by the Examiner, namely column 4, lines 12-21, Bosch states that

> In the case that the DNA is to be introduced into a heterologous organism it may be modified to remove known mRNA instability motifs (such as AT rich regions) and polyadenylation signals, and/or codons which are preferred by the organism into which the recombinant DNA is to be inserted may be used so that expression of the thus modified DNA in the said organism yields substantially similar protein to that obtained by expression of the unmodified recombinant DNA in the organism in which the protein components of the hybrid toxin or toxin fragments are endogenous.

Further, the Examiner states in the Response to Arguments section

> Because Seed and Bosch also teach that AUUUA motifs should be avoided when constructing nucleic acids for expression in heterologous systems, one of skill in the art clearly would have been motivated to remove them from the coding region in view of the teachings of Akashi.

The Bosch reference, however, only generally discloses that DNA encoding a bacterial protein, namely nucleic acids encoding *Bacillus thuringiensis* insecticidal crystal proteins, can be modified by removing instability motifs or using codon optimization to enhance expression in a heterologous organism. This general disclosure of modifying a bacterial protein, however, does not teach or suggest the claimed invention. In particular, there is no teaching or suggestion that a mammalian cell would not express naturally occurring parasitic proteins, or that by replacing codons in a naturally occurring parasitic protein with preferred mammary tissue-specific codons, expression can be obtained in mammary gland cells. In fact, there is no mention whatsoever of mammary gland expression.

Akashi et al. attempt to determine why RNAs coding for either cytokines or oncogenes are unstable and have a short half-life. Specifically, Akashi discloses that the presence of several AUUUA motifs in the 3' untranslated region of a subject hematopoietic growth factor sequence, granulocyte-macrophage colony stimulating factor (GM-CSF) and that they play a role in the stability of these transcripts. Akashi does not address the problems associated with expression of parasitic protein by mammalian cells. Accordingly, Akashi would not teach that the removal of a portion of an mRNA instability motif from the coding region of the parasitic protein allows expression of the protein in mammalian cells. Akashi also does not teach that the lowering of the AT content of nucleic acids encoding parasitic proteins also enhances expression in the mammalian system. Moreover, there is no teaching or suggestion in Akashi to replace a portion

of an mRNA instability motif with a preferred mammary tissue-specific codon, no discussion of mammary gland expression, nor any discussion of producing parasitic proteins in the milk of transgenic mammals. Akashi mentions no other sequences beside oncogenes and nucleic acids encoding hematopoietic growth factors. In the Response to Arguments section, the Examiner states

> Akashi is relied upon in the rejection to show that, at the time of the invention, it was known in the art that AUUUA motifs could destabilize mRNAs even when the motif was located within the coding region.

The Examiner seems to suggest that based on the experiment in Akashi, the effect of AUUUA motifs in the coding region of any gene was known in the art. The experiment suggested that the insertion of a cassette containing seven AUUUA motifs (AT62; see Figure 1A, page 3183, and columns 1 and 2, page 3184) into an exon, and separately, insertion of a cassette of seven AUUUA motifs (AT62) into an intron of RβG, could decrease the stability of RβG RNA. Regarding the results of this experiment, Akashi states, "We cannot rule out that the $t_{1/2}$ of EX2AT62 and EX2GC62 RNA may have been influenced by a change in reading frame, which could effect translation of the RNA." See page 3185, column 2. In view of statements such as these in Akashi, Applicants respectfully submit the experiment performed in Akashi on this point cannot support the Examiner's broad conclusion. For the same reasons discussed with regards to the Dziegiel and Seed references, Applicants submit that Akashi also provides no motivation to arrive at the Applicant's claimed invention.

Thus, the Applicants respectfully submit that Dziegiel, Seed, Akashi, and Bosch, fail to teach, or even suggest, the claimed invention. Moreover, there is nothing in any of these references that suggest that the methods in this large group of publications should or could be combined to arrive at the claimed invention. Of course, it is improper for the Examiner to use applicants' own work as prior art against them as a roadmap to combine other prior art references. It has been clearly established by the Court of Appeals for the Federal Circuit that hindsight analysis is impermissible. See e.g., W.L. Gore & Associates v. Garlock, Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984).

Further, the Applicants submit that their findings were unexpected. Applicants found that the naturally occurring nucleotide sequence encoding parasitic protein MSP-1 was not expressed at detectable levels in mammalian cells, including mammary epithelial cells. Applicants found that by modifying the nucleic acid sequence to replace codons in AT-containing regions and/or mRNA instability motifs with preferred mammary tissue specific codons, high levels of expression of the sequence could be obtained in mammalian cells. Additionally, Applicants discovered that the modified nucleic acid sequence encoding the parasitic protein could be expressed in the milk of a transgenic mammal at levels up to 1 to 2 mg/ml. Clearly, these results were unexpected.

For the reasons discussed above, Applicants submit that the claims are not obvious in view of Dziegiel, Seed, Akashi, and Bosch, and respectfully request that rejection of claims 1-11, 17-21, 23, 24, 27-34, 38-41, and 44-47 under 35 U.S.C. §103 be withdrawn.

CONCLUSION

Applicants submit that all of the claims are now in condition for allowance, which action is requested. Attached is a marked-up version of the changes being made by the current amendment. Enclosed is a check for an Extension of time. Please apply any charges not covered, or any credits, to Deposit Account 06-1050, referencing Attorney Docket No. 10275-134001.

Respectfully submitted,

Date: 7/5/01

Laurie Butler Lawrence
Reg. No. 46,593

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

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Version with markings to show changes made

In the specification:

Paragraph beginning at page 5, line 3, has been amended as follows:

Fig. 1 depicts the cDNA sequence of MSP-1_{42} modified in accordance with the invention [[SEQ ID NO 1]] (SEQ ID NO:1) in which 306 nucleotide positions have been replaced to lower AT content and eliminate mRNA instability motifs while maintaining the same protein amino acid sequence of MSP-1_{42} (SEQ ID NO:9). The large letters indicate nucleotide substitutions.

Paragraph beginning at page 5, line 9, has been amended as follows:

Fig. 2 depicts the nucleotide sequence coding sequence of the "wild type" or native MSP-1_{42} [[SEQ ID NO 2]] (SEQ ID NO:2) and predicted amino acid sequence (SEQ ID NO:10).

Paragraph beginning at page 6, line 12, has been amended as follows:

Fig 6 depicts the nucleic acid sequences of OT1 [[SEQ ID NO 3]](SEQ ID NO:3), OT2 [[SEQ ID NO 4]](SEQ ID NO:4), MSP-8 [[SEQ ID NO 5]](SEQ ID NO:5), MSP-2 [[SEQ ID NO 6]](SEQ ID NO:6), and MSP1 [[SEQ ID NO 7]](SEQ ID NO:7) described in the Examples.

Paragraph beginning at page 6, line 24, has been amended as follows:

Fig 11 is a schematic representation of the nucleotide sequence of MSP42-2 [[SEQ ID NO 8]](SEQ ID NO:8) and predicted amino acid sequence (SEQ ID NO:11).

Paragraph beginning at page 18, line 27, has been amended as follows:

To fuse MSP1-42 to the 15 amino acid β-casein signal peptide, a pair of oligos, MSP203 and MSP204 (MSP203: ggccgctcgacgccaccatgaaggtcctcataattgcctgtctggtggctctggccattgcagcc gtcactccctccgtcat [SEQ ID NO:9](SEQ ID NO:12), MSP204: cgatgacggagggagtgacggctgcaatg gccagagccaccagacaggcaattatgaggaccttcatggtggcgtcgagc (SEQ ID NO:13)) [SEQ ID NO:10], which encode the 15 amino acid - casein signal and the first 5 amino acid of the MSP1-42 ending at the Cla I site, was ligated with a Cla I-Xho I fragment of BC620 (Fig. 8) which encodes the

rest of the MSP1-42 gene, into the Xho I site of the expression vector pCDNA3. A Xho I fragment of this plasmid (GTC669) was then cloned into the Xho I site of milk specific expression vector BC350 to generate B670 (Fig. 9).

Paragraph beginning at page 19, line 8, has been amended as follows:

A Sal I-Not I fragment was prepared from plasmid BC670 and microinjected into the mouse embryo to generate transgenic mice. Transgenic mice [was]were identified by extracting mouse DNA from tail biopsy followed by PCR analysis using oligos GTC17 and MSP101 (sequences of oligos: GTC17, GATTGACAAGTAATACGCTGTTTCCTC (SEQ ID NO:14), Oligo MSP101, GGATTCAATAGATACGG (SEQ ID NO:15)). Milk from the female founder transgenic mice was collected at day 7 and day 9 of lactation, and subjected to western analysis to determine the expression level of MSP-1-42 using [an]a polyclonal anti-MSP antibody and monoclonal [anti MSP]anti-MSP antibody 5.2 (Dr. David Kaslow, NIH). Results indicated that the level of MSP-1-42 expression in the milk of transgenic mice was at 1-2 mg/ml (Fig. 10).

Paragraph beginning at page 19, line 20, has been amended as follows:

Our analysis of the milk produced MSP revealed that the transgenic MSP protein was N-glycosylated. To eliminate the N-glycosylation sites in the MSP1-42 gene, Asn[.] (N) at positions 181 and [262]263 were substituted with Gln[.] (Q). The substitutions were introduced by designing DNA oligos that anneal to the corresponding region of MSP1 and carry the AAC to CAG mutations. These oligos were then used as PCR primers to produce DNA fragments that encode the N to Q substitutions.

Paragraph beginning at page 19, line 27, has been amended as follows:

To introduce N262-Q mutation, a pair of oligos, MSPGYLYCO-3 (CAGGGAATGCTGCAGATCAGC; SEQ ID NO:16) [AND]and MSP42-2 (AATTCTCGAGTTAGTGGTGGTGGTGGTGGTGATCGCAGAAAATACCATG; SEQ ID NO:17, FIG. 11), were used to PCR amplify plasmid GTC627, which contains the synthetic MSP1-42 gene. The PCR product was cloned into pCR2.1 vector (Invitrogen). This generated plasmid GTC716.

Paragraph beginning at page 19, line 33, has been amended as follows:

To introduce N181-Q mutation, oligos MSPGLYCO-1 (CTCCTTGTTCAGG AACTTGTAGGG; SEQ ID NO:18) and MSPGLCO-2 (GTCCTGCAGTACACATATGAG (SEQ ID NO:19), Fig. 4) were used to amplify plasmid GTC 627. The PCR product was cloned into pCR2.1. This generated plasmid GTC700.

In the claims:

Claims 1- 5, 9, 11, 17- 19, 21, 23- 30, and 38-47 have been cancelled.

Claims 6- 8, 10, 20, and 31-37 have been amended as follows:

6. (Amended) A method for producing [preparing a modified nucleic acid encoding] a parasite protein or fragment thereof [for expression] in the milk of a non-human transgenic mammal [a mammalian cell], comprising providing a non-human transgenic mammal whose genome comprises a modified nucleic acid encoding a parasite protein or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified [lowering the AT content of the nucleic acid as it naturally occurs in the parasite] by replacing one or more AT-containing codons of the [naturally occurring] nucleic acid as it naturally occurs in the parasite with a preferred mammary gland [tissue]-specific codon encoding the same amino acid as the replaced codon, and allowing the transgenic mammal to express the parasite protein or fragment thereof in its milk, to thereby produce a parasite protein or fragment thereof.

7. (Amended) A method for producing [preparing a modified nucleic acid encoding] a parasite protein or fragment thereof [for expression] in the milk of a non-human transgenic mammal [in a mammalian cell], comprising: providing a non-human transgenic mammal whose genome comprises a modified nucleic acid encoding a parasite protein or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified [eliminating at least one mRNA instability motif present in the coding sequence of the nucleic acid as it naturally occurs in the parasite] by replacing at least a

portion of an [the]AUUUA mRNA instability motif in the coding sequence as it naturally occurs in the parasite with a preferred mammary gland [tissue]-specific codon encoding the same amino acid as the replaced portion of the AUUUA mRNA instability motif, and allowing the transgenic mammal to express the parasite protein or fragment thereof in its milk, to thereby produce a parasite protein or fragment thereof.

8. (Amended) The method of claim 6 or claim 7, wherein [further comprising replacing] more than one codon in the naturally occurring nucleic acid has been replaced with a preferred mammary gland [tissue]-specific codon encoding the same amino acid as the replaced codon.

10. (Amended) A method for producing [preparing a modified nucleic acid encoding] a parasite protein or fragment thereof [for expression] in the milk of a non-human transgenic mammal [a mammalian cell], comprising [the steps of]:

providing a non-human transgenic mammal whose genome comprises a modified nucleic acid encoding a parasite protein or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified by

a) [eliminating at least one mRNA instability motif present in the coding sequence of the nucleic acid as it naturally occurs in the parasite by] replacing at least a portion of an [the]AUUUA mRNA instability motif in the coding sequence[region] as it naturally occurs in the parasite with a preferred mammary gland [tissue]-specific codon encoding the same amino acid as the replaced portion of the AUUUA mRNA instability motif; and

b) [lowering the AT content of the nucleic acid as it naturally occurs in the parasite by] replacing one or more AT-containing codons of the [naturally occurring] nucleic acid as it naturally occurs in the parasite with a preferred mammary gland [tissue]-specific codon encoding the same amino acid as the replaced codon, and allowing the transgenic mammal to express the parasite protein or fragment thereof in its milk, to thereby produce a parasite protein or fragment thereof.

20. (Amended) A transgenic non-human mammal [animal] whose germline comprises a [the] modified nucleic acid [of claim 4] encoding a parasite protein or fragment thereof operably linked to a promoter which directs expression in the mammary gland, wherein the nucleic acid has been modified by replacing at least a portion of an AUUUA mRNA instability motif in the coding sequence as it naturally occurs in the parasite with a preferred mammary gland-specific codon encoding the same amino acid as the replaced portion of the AUUUA mRNA instability motif and replacing one or more AT-containing codons of the nucleic acid as it naturally occurs in the parasite with a preferred mammary gland-specific codon encoding the same amino acid as the replaced codon, wherein the transgenic mammal expresses the parasite protein or fragment thereof in its milk.

31. (Amended) The method of claim 10, wherein the nucleic acid has the same codon of the naturally occurring nucleic acid replaced with a preferred mammary gland-specific codon such that both the AT content of the naturally occurring nucleic acid is lowered and the mRNA instability motif of the naturally occurring nucleic acid is eliminated by the preferred mammary gland-specific codon [replacing the same codon of the naturally occurring nucleic acid with a preferred mammary tissue-specific codon].

32. (Amended) The method of claim 10, wherein all of the AUUUA mRNA instability motifs present in the naturally occurring nucleic acid have been [are] replaced by a preferred mammary gland [tissue]-specific codon.

33. (Amended) The method of claim 10, wherein the modified nucleic acid further comprises at least one additional codon other than the codon replaced to lower AT content or the codon replaced to eliminate an mRNA instability motif which has been [is] replaced with a preferred mammary gland [tissue]-specific codon.

34. (Amended) The method of claim 10, wherein all of the codons of the naturally occurring nucleic acid have been [are] replaced with a preferred mammary gland [tissue]-specific codon.

35. (Amended) The method of claim 10, wherein the modified nucleic acid is expressed in milk [by the mammalian cell] at a level which is at least 25% more than the naturally occurring nucleic acid is expressed under the same conditions [by the same type of mammalian cell].

36. (Amended) The method of claim 10, wherein the modified nucleic acid is expressed in milk [by the mammalian cell] at a level which is at least 50% more than the naturally occurring nucleic acid is expressed under the same conditions [by the same type of mammalian cell].

37. (Amended) The method of claim 10, wherein the modified nucleic acid is expressed in milk [by the mammalian cell] at a level which is at least 100% more than the naturally occurring nucleic acid is expressed under the same conditions [by the same type of mammalian cell].

Please add claim 48 as follows:

48. (New) The method of claim 10, wherein all non-preferred mammary gland codons are replaced with preferred mammary gland specific codons.